PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
21581 WO-BUR		
International application No.	International filing date *(day/month/year)*	Priority date *(day/month/year)*
PCT/EP2004/000729	28.01.2004	29.01.2003
International Patent Classification (IPC) or both national classification and IPC		
C07H21/04		
Applicant		
ROCHE DIAGNOSTICS GMBH et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets, including this cover sheet.

   ☒ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 2 sheets.

3. This report contains indications relating to the following items:

   - I ☒ Basis of the opinion
   - II ☐ Priority
   - III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
   - IV ☐ Lack of unity of invention
   - V ☒ Reasoned statement under Rule 66.2(a)(ii) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
   - VI ☐ Certain documents cited
   - VII ☐ Certain defects in the international application
   - VIII ☐ Certain observations on the international application

Date of submission of the demand	Date of completion of this report
09.06.2004	29.11.2004
Name and mailing address of the international preliminary examining authority:	Authorized Officer
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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/EP2004/000729

I. Basis of the report

1. With regard to the **elements** of the international application *(Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17))*:

Description, Pages

1-26 as originally filed

Claims, Numbers

1-14 received on 07.09.2004 with letter of 03.09.2004

Drawings, Sheets

1/14-14/14 as originally filed

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.

These elements were available or furnished to this Authority in the following language: , which is:

- ☐ the language of a translation furnished for the purposes of the international search (under Rule 23.1(b)).
- ☐ the language of publication of the international application (under Rule 48.3(b)).
- ☐ the language of a translation furnished for the purposes of international preliminary examination (under Rule 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

- ☐ contained in the international application in written form.
- ☐ filed together with the international application in computer readable form.
- ☐ furnished subsequently to this Authority in written form.
- ☐ furnished subsequently to this Authority in computer readable form.
- ☐ The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.
- ☐ The statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished.

4. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

5. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)).

   (Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.)

6. Additional observations, if necessary:

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-14
	No:	Claims	None
Inventive step (IS)	Yes:	Claims	1-14
	No:	Claims	None
Industrial applicability (IA)	Yes:	Claims	1-14
	No:	Claims	None

2. Citations and explanations

   see separate sheet

Re Item V

Reasoned statement with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. The following documents have been used in the evaluation of the present application:
   D1: NUCLEIC ACIDS RESEARCH, vol. 29, no. 13, 2001, pages e65-1-e65-7
   D2: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, vol. 92, no. 3, 1970, pages 724-726
   D3: ANALYTICAL BIOCHEMISTRY, vol. 226, 1995, pages 161-166
   D4: THE JOURNAL O BIOLOGICAL CHEMISTRY, vol. 257, no. 9, 1982, pages 4796-4805
   D5: BIOTECHNIQUES, vol. 33, no. 3, September 2002, pages 526-531
   D6: NUCLEIC ACIDS RESEARCH, vol. 22, no. 4, 1994, pages 695-696
   D7: NUCLEIC ACIDS RESEARCH, vol. 26, no. 21, 1998 pages 5009-5010
   D8: NUCLEIC ACIDS RESEARCH, vol. 22, no. 15, 1994, pages 2990-2997
   D9: US-A-4 844 880

2. **Novelty (Article 33(2) PCT):**
   The claimed subject-matter of the newly filed **claims 1-14** of the present application are not disclosed in the documents cited and is therefore considered novel. These claims fulfil the requirements of **article 33(2) PCT**.

3. **Inventive merit (Article 33(3) PCT):**
   D1, which is considered to be the closest prior art, concerns the transformation of cytosine into uracil using various operating conditions involving bisulphite as a reactant (see page e65-2, "deamination"; page e65-3, table 1 and last paragraph). In particular, this document describes the bisulphite reaction at 80 and 85 degrees Celsius during 1 and 4 hours (among others) and using bisulphite concentrations between 3.87 - 4.26 M or between 5.20 - 5.69 M at pH 5.0. This document also clearly teaches that by increasing the reaction temperature, the full conversion is achieved in a shorter time.
   The method of the application distinguishes itself from **D1** by the reacting time which is between 1.5 and 3.5 hours.
   From the comparative tests provided by the applicant (with letter of 13.10.2004) it appears that the technical effect achieved by selecting a reaction time between 1.5 and 3.5 hours using the concentration, pH and temperature as defined in **claim 1** is that a higher transformation yield is obtained.

The problem to be solved by the present application can therefore be formulated as to find a method to transform cytidine into uracil with better yield.

The solution suggested by the present application is therefore an alternative to **D1**.

The comparative example presented in the tests of 13.10.2004 demonstrate the unexpected effect that the combination of the specific conditions (concentration of bisulfite, pH, temperature and reaction time) give a higher transformation yield.

Due to this unexpected result, an inventive merit can be recognised in the method of **claim 1** which thus fulfills the requirements of **article 33(3) PCT**.

The optimised conversion conditions being obtained by the combination of the appropriate concentration of bisulfite, pH and the temperature, the use of such a solution for the conversion of cytosine to uracil (**claim 8**) as well as the kit (**claim 11**) and the solution (**claim 12**) claimed are also considered to demonstrate an inventive merit over the prior art.

It is concluded that **claims 1-14** of the present application fulfil the requirements of **article 33(3) PCT**.

4. **Industrial applicability (Article 33(4) PCT):**

Due to the nature of the claims, an industrial applicability of the invention is obvious and **claims 1-14** are considered to fulfil the requirements of **Article 33(4) PCT**.